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HPLC-UV Method for the Analysis of Potato Sprout Inhibitor Chlorpropham and Its Metabolite 3-Chloroaniline in Potatoes

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Abstract: *A simple extraction method based on methanol-soaking coupled with HPLC-UV detection was developed for the simultaneous determination of the potato sprout inhibitor chlorpropham (isopropyl 3-chlorophenyl carbamate) (CIPC) and its degradation product 3-chloroaniline (3-CA) in potato samples using propham (IPC) as an internal standard. The chromatographic conditions selected were 62% methanol at 15 minutes run time and retention times at 11 and 4 minutes for CIPC and 3-CA respectively. The HPLC method was validated reporting good precision and acceptable linearity. The estimated values of LOQ in the organic potato extract based on fresh potato tuber weight were 0.01 and 0.02 mg/kg of CIPC and 3-CA respectively.*
The initial extraction using overnight methanol-soaking gave recovery values of up to 90% for CIPC following spiking organic potato peel for 1 hour at spiking levels of 0.8 and 8.0 µg/g. However, 3-CA recovery was found to be very low (< 10%) particularly at the lower level of 0.8 µg/g when no 3-CA was detected. To improve the extractability of 3-CA, the extracting solution of methanol was acidified by adding sulphuric acid. Optimising the extraction process showed that using 1 M sulphuric acid prepared in 50% methanol as an extracting solution for 24 hours at 50 ºC improved the extraction recovery of 3-CA up to 85% at a spiking level of 8.0 µg/g and contact time 1 hour. This final extraction method was applied for the determination of the residues of both CIPC and 3-CA in commercial potato samples which had received several applications of CIPC reporting high residue values of 3-CA whereas CIPC residues were lower than maximum residue level (MRL) of 10 mg/kg.
Keywords: *Potato, Chlorpropham; 3-Chloroaniline; Sprout inhibitor; HPLC*

I. Introduction

Chlorpropham (CIPC) is the main sprout inhibitor currently used by potato industry worldwide. Application of CIPC by thermal fogging in potato stores may cause thermal degradation of CIPC. The high temperature of the fogging machine which most often ranges from 300 to 600 ºC and contact of CIPC with hot surfaces such as the aluminum pipe used to carry the fog into the store can promote thermal degradation [1,2]. A study of CIPC in the atmosphere of potato stores treated with CIPC showed the presence of another compound, which was later identified as 3-chloroaniline (3-CA) [1] . The thermostability of CIPC was investigated by Smith et al. [3] who concluded that CIPC is predicted to be unstable under the conditions of its application. Decomposition of chlorpropham is predicted to occur via ester pyrolysis at $T > 500$ °C. Highly toxic of 3-CA is the final product of CIPC decomposition. In addition to thermal degradation, the temperature of the stored potatoes (typically 9 - 10 for processed potatoes ^{o}C) and moisture may encourage bacterial growth, causing microbial degradation of CIPC. Several studies have been reported on the microbial degradation of CIPC which leads to the formation 3-CA [4- 6]. After application of CIPC to potatoes in stores, residues of CIPC and its metabolite 3-CA have been identified in treated potatoes after long term storage [7,8]. These residues remaining in the potato tubers are of concern for consumers due to their possible toxicity particularly 3-CA which is an aromatic amine and is known to be dangerous to humans and the environment [9]. The determination of CIPC and its metabolite 3-CA in potato samples is receiving increasing attention by the potato industry. A document (SANCO-4967-2009-rev-3) relating to European Communities Commission regulations set out the foods to be sampled and the product/pesticide combinations to be tested during the years 2010, 2011 and 2012. The text related to chlorpropham stated that chlorpropham and 3-chloroaniline should be combined and expressed as chlorpropham. It was recommended that the MRL for CIPC was to be 10 mg/kg in potato samples and this should include its metabolite 3-CA [10]. Therefore, determination of their levels in potatoes is very important for the potato processing industry and human consumption, thus, the focus has been towards developing suitable analytical methods permitting good extraction and interference free quantification that can be applied to potato samples.

In reviewing the literature, few documented methods have focused on the determination of CIPC in combination with its degradation products specifically 3-CA in potato samples. Most of these methods involved homogenisation and extraction with an organic solvent (e.g. methanol, petroleum ether/acetone, hexane/acetone) followed by partition into dichloromethane. After purification, the extracts were transferred into a volatile solvent and determination was carried out using gas liquid chromatography. The recovery data from spiking whole potato

and fresh peel was found to be quite variable ranging from 36 – 128% for CIPC and from 0 – 77% for 3-CA [7]. In addition, aniline metabolites of CIPC have been identified in potato samples using micellar electrokintic chromatography coupled with laser induced fluorescence detection [11].

HPLC seems to be more appropriate to determine carbamate pesticides residues mainly to overcome the thermal lability problems and derivatisation step when using other techniques [12-16]. However, no HPLC method have included the analysis of CIPC in combination with its degradation products in particular 3-CA in potato samples.

The main objective of this work was to develop an analytical method based on a suitable extraction procedure coupled with HPLC-UV for the determination of parent pesticide CIPC and its degradation product 3-CA in potato samples.

II. Materials And Methods

2.1 Materials and standards

Chlorpropham (95%), 3-chloroaniline (99%) and propham (IPC) (99%) were purchased from Sigma-Aldrich Chemi GmbH (Germany), methanol (HPLC grade), sulphuric acid and sodium hydroxide were supplied by Fisher Scientific International Company (UK). Stock solutions of 10000 µg mL^{-1} of each compound (CIPC, IPC and 3-CA) were prepared in methanol. These individual stock standard solutions were stored in a refrigerator at 4 °C and used to prepare the working solutions at different concentrations.

2.2 HPLC system

The HPLC system used comprised a GILSON® 234-auto sampler, Cecil 1100 Series pump, Phenomenex® Security Guard™ (part no. KJO-4282) guard column with analytical column Phenomenex® (ODS-2 250 mm x 4.6 mm 5 µm Sphereclone), and a Thermo Separation SpectraSERIES UV100 detector coupled with Dionex Peaknet software. A column oven (LaChrom, Merck L-7350) was connected with a cooling system (Techne, Tecam® R 4-2) to control the column temperature at 25 ºC. The water used for preparation of the mobile phase was supplied from an Elga Purelab Option deioniser model LA613, then filtered through a Supor®-200 membrane filter (47 mm 0.2 µm). The mobile phase was degassed using an ultrasonic bath (Camlab CamSonix C425). All analyses were performed at a detection wavelength of 210 nm, pump flow rate of 1.5 mL/min and injection volume of 20 µL.

2.3 Optimisation and validation HPLC analytical method

The effect of the different concentrations of the mobile phase (70%, 65%, 62%, 60%, 55% and 50% methanol) were investigated to optimise the separation of intended compounds and construct a basic background for developing an HPLC separation method with high resolution and rapid analysis of the eluted compounds.

The HPLC analytical method was validated by measuring precision, linearity and LOD and LOQ. To measure the precision, five replicate injections of 1 µg/mL of a mixture CIPC, IPC and 3-CA were injected. The linearity of the calibration curve was tested at three ranges of concentrations (0.02 – 0.1, 0.2 – 1.0 and 2 – 10 µg/mL) prepared as three series of standard solutions of a mixture CIPC, IPC and 3-CA in methanol. The LOD and LOQ were estimated for three compounds through statistical data from plotting the calibration curve at low concentration range of 0.02 – 0.1 µg/mL (LOD= 3SD/slope and LOQ= 10SD/slope).

2.4 Soaking extraction

Sample preparation involved washing potato tubers for two minutes under cool running tap water to eliminate the soil and any CIPC that may be adsorbed on to the soil. After air-drying, the weight of each potato tuber was recorded using a top pan balance, each tuber was peeled with a stainless steel peeler and the weight of the total peel was recorded. The peel was chopped into fine pieces and carefully mixed to obtain good homogeneity. A subsample of 2.5 g of chopped peel sample was weighed into a 50 mL screw top jar, then 20 mL methanol containing the internal standard of 1 µg mL^{-1} propham (IPC) was added as the extracting solution. The samples were left soaking overnight extraction (~16 hours) at room temperature. The extract was filtered and transferred into HPLC vials through a 0.2 µm PTFE (Teflon) membrane syringe filter prior to analysis.

2.5 Acid-methanol soaking extraction

Acid-methanol soaking extraction involved using an extracting solution of 1 M H_2SO_4 prepared in 50% methanol containing an internal standard of IPC for an extraction period of 24 hours at 50 °C. 2 mL of the extract was neutralised with a suitable volume of 1 M NaOH (pH ~ 3–5 to maintain the efficiency and stability of the HPLC column), and made up to 5 mL with methanol. The extract was filtered through a 0.2 µm PTFE membrane syringe prior to transfer into an HPLC vial for analysis.

2.6 Limit of quantification of the studied compounds in the organic potato extract

The limit of quantification (LOQ) for the studied compounds in potato extract were estimated by replicate injections (n = 10) of a 0.05 µg/mL mixture of CIPC, IPC and 3-CA prepared in an extract of organic potato.

2.7 Recoveries of compounds from spiking organic potato peel

The methanol-soaking extraction coupled with HPLC analysis was applied to measure the recovery of CIPC and 3-CA. 2.5 g (n=5) of organic potato peel was spiked with 100 µL of spiking solution containing CIPC and 3-CA at spiking levels of 0.8 and 8.0 µg/g. The spiked samples were left for 1 hour prior to extraction with 20 mL of methanol (containing IPC as the internal standard) for approximately 16 hours. Additionally, replicates (n=5) of a control without peel were carried out where 100 µL of spiking solution was added directly to empty bottles which were sealed for 1 hour prior to carrying out the same extraction. The effect of the spiking time on the recovery of 3-CA was also studied for different periods of time (0, 3, 5 and 60 minutes) at spiking level of 8.0 µg/g.

2.8 Optimisation the analytical method for extraction of 3-CA

Sulphuric acid combined with methanol was used to extract 3-CA from the spiked potato peel preparing 1 M of this acid in methanol at different percentages (0, 10, 25, 50 and 75). The influence of temperature and extraction time on the extraction recovery of 3-CA using 1 M sulphuric acid in 50% methanol were also tested.

The degradation of CIPC by acid hydrolysis under the extraction conditions was investigated. A solution of 10 µg mL^{-1} CIPC was prepared in the extracting solution of 1 M sulphuric acid in 50% methanol and kept at the extraction temperature of 50 ºC for 24 hours. The analysis of this solution was compared with a mixed standard solution of 10 µg mL^{-1} of 3-CA, IPC and CIPC prepared in a mixture of 1 M sulphuric acid in 50% methanol at ambient temperature.

2.9 Determination of the residues of CIPC and 3-CA in commercial samples

The proposed analytical method was tested on potatoes tubers treated with CIPC from a commercial potato store to determine the residues of both CIPC and 3-CA. The proposed method was compared with using methanol alone as extracting solution. Randomly, 20 potato tubers were selected from bags which were obtained from UK stores that had received CIPC application. A subsample was taken for each extraction.

III. Results And Discussion

3.1 HPLC analysis

To measure CIPC and 3-CA from their mixture solution the chromatographic conditions were set based on an isocratic method using methanol/water as the mobile phase. Propham (IPC) was chosen as the internal standard due to its similarity in structure to chlorpropham with the only difference being the absence of one chlorine atom in the phenyl ring. Optimising the chromatographic conditions produced good separation of compounds from the mixture (Fig 1) selected 62% methanol at a 15 minutes run time. The retention times of CIPC and 3-CA were 11 and 4 minutes respectively. No interferences at the retention time of 3-CA was confirmed by analysis an extract of spiked potato peel (with 3-CA and internal standard excluding CIPC) and extract of control potato peel using low eluant strength (55%) as shown in Fig 2.

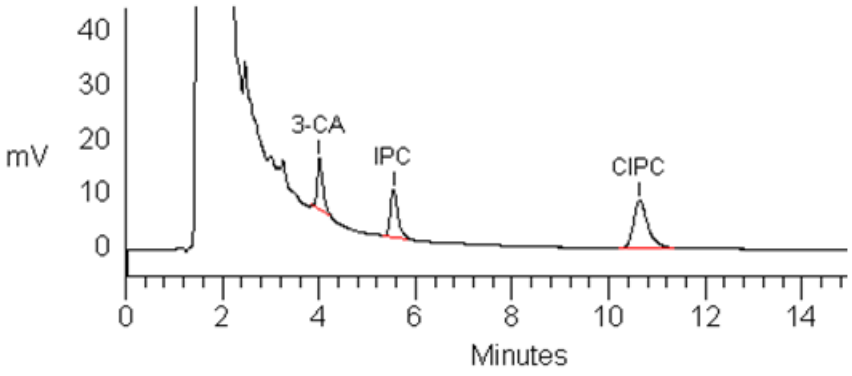

Fig. 1 Sample HPLC chromatogram of potato peel extracted with 1 M H_2SO_4 in 50% MeOH at 50 °C.

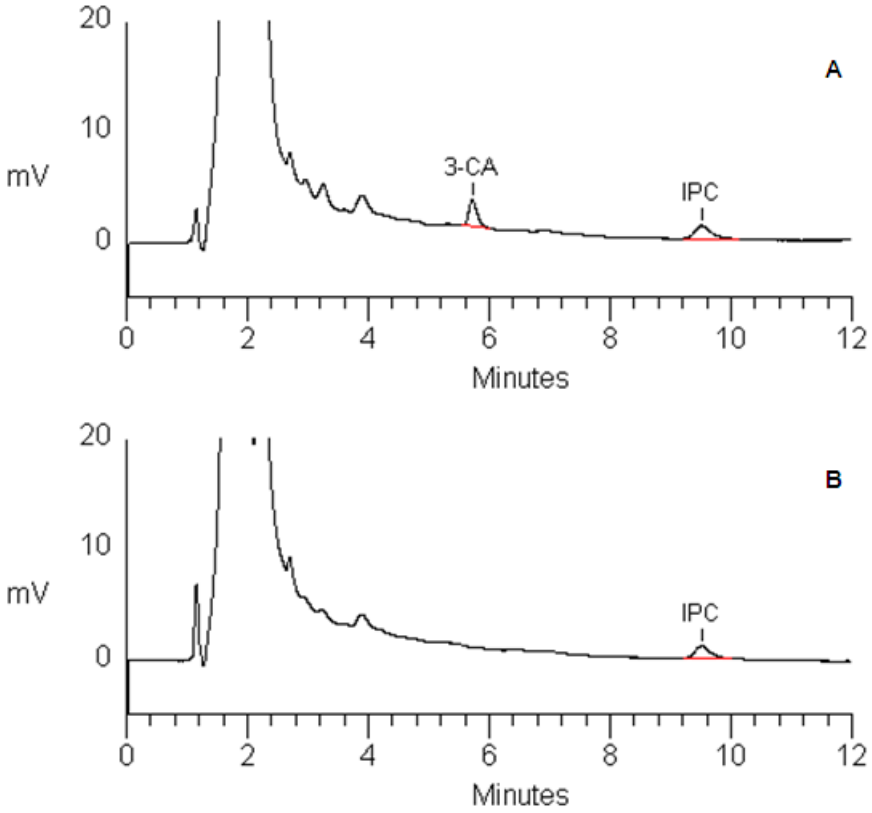

Fig. 2 Sample HPLC chromatograms at 55% mobile phase of the extract of a- spiked potato peel with 3-CA and internal standard b- Control potato peel.

The HPLC method was validated confirming good precision (RSD% < 0.03), acceptable linearity (R^2 > 0.990) and low values of LOD (0.01 µg/mL) and LOQ (0.04 µg/mL) for both CIPC and 3-CA in standard solutions.

3.2 Limit of quantification of the studied compounds in the organic potato extract

The estimated values of LOQ in the organic potato extract based on fresh potato tuber weight were 0.01 and 0.02 mg/kg of CIPC and 3-CA respectively. Generally, these values are acceptable for the quantitative determination of CIPC and 3-CA residues at low levels in potato peel extract. No clean up step was required other than filtration, however, to obtain lower values for the LOQ a further clean up may be useful**.**

3.3 The recoveries of compounds from spiking organic potato peel

In order to evaluate the efficiency and the accuracy of the extraction method using overnight methanol-soaking coupled with HPLC-UV, the recovery measurement of studied compounds in potato samples was investigated. Recovery values represent the ratio of the amounts extracted and measured from the total amount added of solution of CIPC and 3-CA at spiking levels of 0.8 and 8.0 µg/g to organic potato peel after a contact time of 1 hour. The recoveries obtained from spiked peels are up to 90% for CIPC with acceptable RSD% (< 10%) for five replicates at the two spiking levels. In contrast, the recovery of 3-CA from spiking potato peel was found to be 10% (RSD%= 21%, n= 5). No peak was detected for 3-CA at the lower level of 0.8 µg/g compared to the high recoveries of control (no peel) which were greater than 93% (RSD% < 11%, n= 5) at the two spiking levels. The high recovery from the control confirmed that no concern of volatilisation or adsorption of 3-CA onto the glass container.

The low recovery of 3-CA can be attributed to incomplete extraction of non-extractable bound residues within the potato peel matrix. It is well known that plants can incorporate pesticides and their metabolites into bound and non-extractable residues. These residues resist solubilisation in common solvents and are therefore not accessible to standard residue analysis [17]. The non-extraction of the chemical residue from the sample matrix depends on its chemical properties and reactive functional groups, time course of binding, environmental factors influencing binding rates, binding sites and mechanisms and the extraction procedure [18,19]. 3-CA is an aromatic amine, the quantitative determination of this group of compounds from different environmental matrices generally provides an analytical challenge associated with low extraction recoveries and difficult separation chromatography due to the physicochemical properties of volatility, polarity, basicity and high water solubility [20]. The

understanding of this binding process and non-extractable residues is not clear due to the complex structure of the potato peel matrix. However, the reason for the poor extraction of 3-CA from potato peel might be strong binding by ion exchange, Schiff base reaction and hydrogen bonding with potato components or enzymatic activity.

The spiking time has an important role of the extractability. Studying the effect of the spiking time for different periods of time (0, 3, 5 and 60 minutes) showed a clear trend of decreasing recovery of 3-CA as shown in Fig 3.

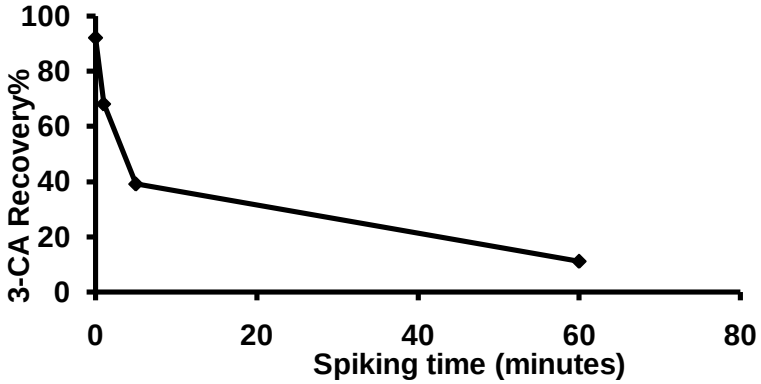

Fig. 3 The effect of spiking time on the recovery of 3-CA from potato peel (n=5) at spiking level of 8.0 µg/g.

The longer the time between addition of the spike and addition of the extractant, the more difficult it becomes to extract 3-CA from the peel. It should be noted that the recovery of an analyte from spiking the matrix under laboratory conditions for known times is unlike real samples under commercial store conditions. In spiked samples, the analyte may well not reach equilibrium whereas the analyte in commercial samples may do, taking into account the long time between application and analysis. Thus, the recovery from treated potato samples is expected to be lower than that from spiking organic peel samples.

3.4 Optimisation the method for extraction of 3-CA

It was observed that the highest recovery of 3-CA onto the potato peel in aqueous solution, occurred at low pH [21]. Using high acidity of the extracting solution seemed to have a considerable effect in enhancing the extraction of 3-CA from potato peel. As can be seen in Fig 4, it seems that the strong acidity in the extractant is responsible for improving the extraction from potato peel. The recovery of 3-CA is not affected by increasing the methanol percentage in the extractant showing recovery values up to 60% in an acidic solution of methanol at all percentages of methanol at ambient temperature compared to poor recovery (10%) when using methanol alone at spiking level 8.0 µg/g.

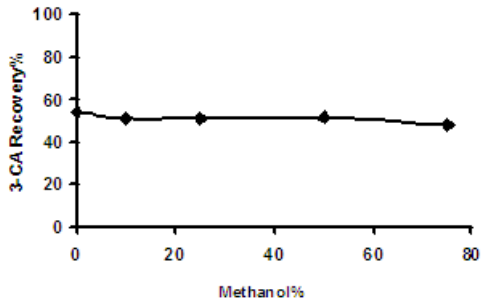

Fig. 4 Recoveries of 3-CA from spiking potato peel using extracting solution of 1 M H_2SO_4 in different percentages of methanol at ambient temperature.

The main purpose of mixing methanol with sulphuric acid is that the organic solvent can wet the surface of the potato peel and penetrate the potato substrates allowing sulphuric acid to break the interaction between the potato peel and the 3-CA. As the objective of this study is to extract both residues of 3-CA and CIPC in a potato sample extract, a high concentration of methanol is required to extract the CIPC. Therefore, 50% methanol was chosen and used for optimising the extraction process and investigation other parameters of temperature and extraction time.

Extraction temperature is one of the essential factors for optimising the extraction process. It affects the mass transfer rates of the analyte from the matrix to the extraction solution. The effect of temperature on the extraction efficiency of 3-CA from spiked peel using an extracting solution of 1 M sulphuric acid in 50% methanol was investigated at different temperatures (ambient, 22, 50 and 70 °C) for overnight extraction. Recovery data for 3-CA in Fig 5 shows that the extraction recovery increased with increasing temperature.

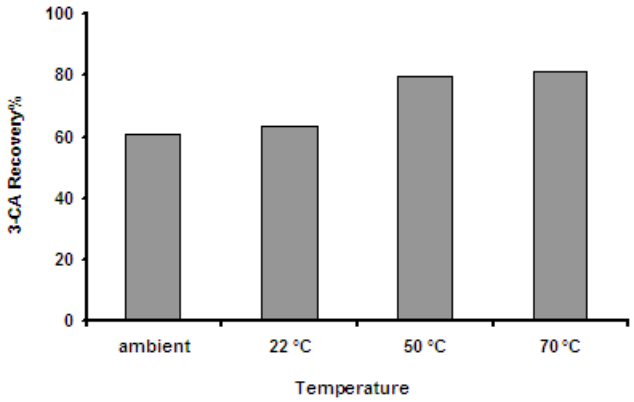

Fig. 5 The effect of temperature on the recovery of 3-CA from potato peel extracted overnight with a solution of 1 M H_2SO_4 in 50% methanol.

The high temperature might decrease the binding strength of 3-CA with the potato peel and subsequently increase the distribution rate of 3-CA into the extractant thus increasing the extraction efficiency. The best recoveries were obtained at 50 °C and 70 °C up to 82 %. As there was a little difference in the extraction efficiency between these two temperatures, 50 °C was selected in this study.

The extraction time is another essential factor to be optimised in an extraction procedure. Mostly, the extraction recovery of analytes increases with increasing extraction time until reaching an equilibrium, because the longer time allows more contact between the extracting solvent and sample matrices. However, it is not always practical to use an extraction time that is long enough for equilibrium to be achieved [22]. To establish the optimal conditions for the extraction procedure of 3-CA using an extracting solution of 1 M H_2SO_4 in 50% methanol, the extraction of replicate spiked samples was performed in the incubator at 50 ºC over a ranged of different extraction times (2, 6, 12, 18 and 24 hours). Fig 6 shows that the extraction recovery of 3-CA increased with extraction time.

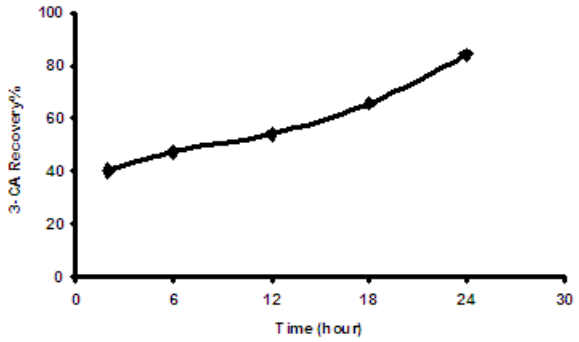

Fig. 6 Effect of the extraction time on the extraction recovery of 3-CA using the extracting solution of 1 M H_2SO_4 in 50% methanol at 50 °C.

Even though the extraction did not reach equilibration at the longest time of 24 hours, the best extraction was achieved at 24 hours extraction time where the recovery was found of up to 85%. Although, a higher recovery value may be obtained if an extraction time of greater than 24 hours is used but this extraction time of 24 hours was considered a reasonable and an acceptable time for extraction procedure of 3-CA from potato samples.

CIPC is a compound of the well known group of N-phenyl carbamates which may udergo rapid degradation under unsuitable solvent and excessive heating conditions [23]. CIPC can be hydrolysed under acidic or alkaline conditions, releasing 3-CA [6,24-25]. To investigate the possible hydrolysis of CIPC to form 3-CA, CIPC solution was tested under the same conditions as used for the extraction of 3-CA and showed no degradation.

3.5 Determination of the residues of CIPC and 3-CA in commercial samples

To check the extraction method using a mixture of 1 M H_2SO_4 in 50% methanol at 50 °C for 24 hours coupled with HPLC-UV analysis, potatoes tubers treated with CIPC from a commercial store were analysed to determine the residues of CIPC and 3-CA. In addition, comparisons were made with extraction using methanol alone at ambient temperature. The results obtained from the analysis of the residue values of 3-CA and CIPC in the 20 potato tubers are shown in Fig 7 and 8.

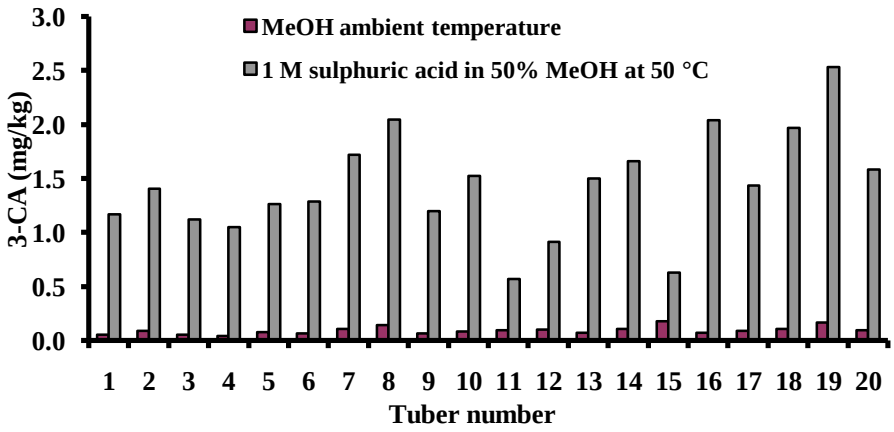

Fig. 7 The residue of 3-CA in 20 treated potato tubers extracted by two extractants.

The histogram in Fig 7 indicates that there is a clear trend of a higher residue concentration of 3-CA in all 20 potato tubers using the proposed method of 1 M H_2SO_4 in 50% MeOH at 50 °C for 24 hours compared with methanol alone at ambient temperature (mean residue values were 1.43 and 0.09 mg/kg respectively).

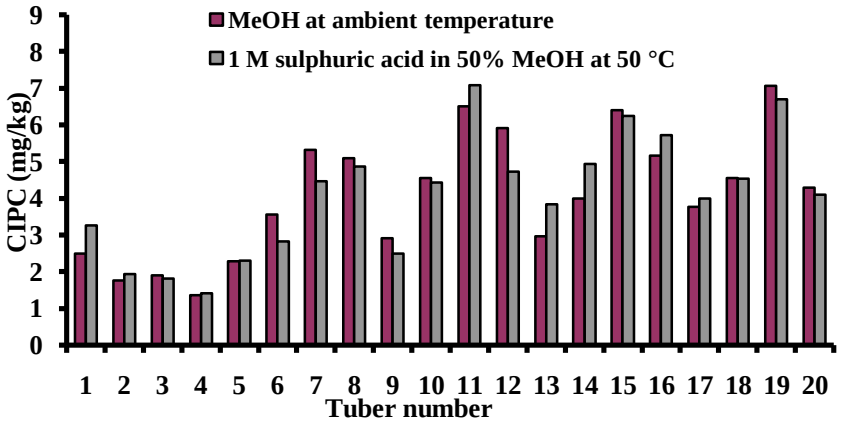

Fig. 8 The residue of CIPC in 20 treated potato tubers extracted by two extractants.

The mean CIPC residue value determined for these 20 potato tubers was the same (4.09 mg/kg) for both extractions. Further statistical analysis using a paired t-test showed that there was no significant difference ($p > 0.05$) between the two extractants used to extract the residue of CIPC in 20 treated tubers. The conclusion that can be drawn is that the acid soaking extraction method was suitable for extraction of the residue of CIPC as well as that of 3-CA.

It can be seen from the data in Fig 7 and 8 that the most striking result was that a high residue concentration of 3-CA (0.57–2.53 mg/kg) was detected in these potato samples whereas CIPC residues were lower than maximum residue level (MRL) of 10 mg/kg. It seems possible that this residue of 3-CA is due to degradation of CIPC during application in the store. CIPC was applied to the potato tubers as a solid formulation, melting at 37 ºC and fogged at 450 ºC through metal pipes. Degradation of CIPC might occur due to pyrolysis on contact with metal surfaces at high temperatures resulting in the formation of 3-CA. These potatoes were analysed at the end of the season, meaning that they may have received several applications of CIPC. Another possible reason for this residue of 3-CA is that microbial degradation of CIPC residue might have occurred during the long storage period. Furthermore, 3-CA is used to synthesise CIPC commercially by reacting with isopropylchloroformate so it may be present in the CIPC formulation as a contamination [7,2].

IV. Conclusion

No suitable analytical method for the simultaneous determination of the potato sprout inhibitor chlorpropham and its major metabolite 3-chloroaniline in potatoes was documented in the literature. Methanol-soaking overnight extraction (~ 16 hours) coupled with HPLC-UV was developed for the determination of CIPC in potato peel showing high recovery of up to 90%, but initially with poor extraction of 3-CA. Changing of the pH of the extracting solution indicated that high acidity using sulphuric acid combined with methanol improved the extractability of 3-CA. The extraction process was optimised for methanol concentration, temperature and extracting time showing that using a mixture of 1 M H_2SO_4 in 50% methanol as an extracting solution for 24 hours at 50 °C increased the extraction recovery of 3-CA up to 85%. This procedure represents a straightforward and acceptable method for the extraction and analysis of 3-CA from potato peel samples and furthermore it can be used for the simultaneous extraction of CIPC.

References

[1]. L.J. Park, Chlorpropham distribution in potato stores and evaluation of environmental issues relating to its use,Ph.D. Thesis, University of Glasgow, 2004, http://theses.gla.ac.uk/4402/.

[2]. L. Park, H. Duncan, A. Briddon, A. Jina, A. Cunnington, and S. Saunders, Review and development of the CIPC application process and evaluation of environmental issues, AHDB-Potato Council, 2009, http://www.potato.org.uk.

[3]. M. J. Smith, S. Müller, W. Sander, and G. Bucher, Mechanisms of the thermal decay of chlorpropham, Journal of Hazardous Materials, 246-247, 2013,154-162.

[4]. P. Verhagen, L. De Gelder, S. Hoefman, P. De Vos, and N. Boon, Planktonic versus biofilm catabolic communities: Importance of the biofilm for species selection and pesticide degradation, Applied and Environmental Microbiology, 77**,** 2011, 4728-4735.

[5]. D.D. Kaufman and P.C. Kearney, Microbial degradation of isopropyl-n-3-chlorophenylcarbamate and 2-chloroethyl-n-3-chlorophenylcarbamate, Applied Microbiology, 13**,** 1965, 443-446.

[6]. P.C. Kearney and D.D. Kaufman, Enzyme from soil bacterium hydrolyzes phenylcarbamate herbicides, Science, 147**,** 1965, 740-741.

[7]. B.L. Worobey and W.F. Sun, Isolation and identification of chlorpropham and 2 of its metabolites in potatoes by GC-MS, Chemosphere, 16**,** 1987, 1457-1462.

[8]. G. Mcgowan, H. Duncan, A. Briddon, and A. Cunnington, Review of levy-funded research on chlorpropham (CIPC), AHDB-Potato Council, 2010, http://www.potato.org.uk.

[9]. M. Sihtmaee, M. Mortimer, A. Kahru, and I. Blinova, Toxicity of five anilines to crustaceans, protozoa and bacteria, Journal of the Serbian Chemical Society, 75, 2010, 1291-1302.

[10]. M.J. Smith and G. Bucher, Tools to study the degradation and loss of the N-phenyl carbamate chlorpropham - A comprehensive review, Environment International, 49, 2012, 38-50.

[11]. E. Orejuela and M. Silva, Rapid determination of aniline metabolites of chlorpropham in potatoes by micellar electrokinetic chromatography using negative-charged mixed micelles and laser-induced fluorescence detection, Electrophoresis, 26**,** 2005, 2991-2998.

[12]. H. Lee, M. Riu, E. Kim, J. K. Moon, H. Choi, J. A. Do, J. H. Oh, K. S. Kwon, Y. D. Lee, and J. H. Kim, A single residue method for the determination of chlorpropham in representative crops using high performance liquid chromatography, Journal of the Korean Society for Applied Biological Chemistry, 56 (2), 2013, 181-186.

[13]. M. Koniger and P. R. Wallnofer, Fate of sprout inhibitors propham (IPC) and chlorpropham (CIPC) in potatoes in relation to different cooking techniques, Deutsche Lebensmittel-Rundschau, 94**,** 1998, 229-231.

[14]. A. S. Arribas, E. Bermejo, M. Chicharro, and A. Zapardiel, Application of matrix solid-phase dispersion to the propham and maleic hydrazide determination in potatoes by differential pulse voltammetry and HPLC, Talanta, 71**,** 2007, 430-436.

[15]. A. M. Wilson, A. A. Bushway, and R. J. Bushway, Residue analysis of isopropyl n-(3-chlorophenyl) carbamate in fruits and vegetables using high-performance liquid-chromatography, Journal of Agricultural and Food Chemistry, 29**,** 1981, 746-749.

[16]. E. Orejuela and M. Silva, Determination of propham and chlorpropham in postharvest-treated potatoes by liquid chromatography with peroxyoxalate chemiluminescence detection, Analytical Letters, 37**,** 2004, 2531-2543.

[17]. H. Sandermann, Bound and unextractable pesticidal plant residues: Chemical characterization and consumer exposure, Pest Management Science, 60**,** 2004, 613-623.

[18]. M. W. Skidmore, G.D. Paulson, H. A. Kuiper, B. Ohlin, and S. Reynolds, Bound xenobiotic residues in food commodities of plant and animal origin, Pest Management Science, 58 **,** 2002, 313-313.

[19]. T. R. Roberts, IUPAC reports on pesticides .17. Non-extractable pesticide-residues in soils and plants, Pure and Applied Chemistry, 56**,** 1984, 945-956.

[20]. T. S. Oostdyk, R. L. Grob, J. L. Snyder, and M. E. Mcnally, Study of sonication and supercritical fluid extraction of primary aromatic-amines, Analytical Chemistry, 65**,** 1993, 596-600.

[21]. N. M. Sher Mohammed, Extraction and HPLC analysis of potato sprout suppressant chemicals, Ph.D. Thesis, University of Glasgow, 2012, http://theses.gla.ac.uk/3454/.

[22]. Q. Zhou and C. Ye, Ionic liquid for improved single-drop microextraction of aromatic amines in water samples, Microchimica Acta, 162**,** 2008, 153-159.

[23]. C. Przybylski and V. Bonnet, Combination of ^{1}H nuclear magnetic resonance spectroscopy and mass spectrometry as tools for investigation of the thermolytic and solvolytic effects case of carbamates analysis, Journal of Chromatography A, 1216**,** 2009, 4787-4797.

[24]. J. Hajslova and J. Davidek, Indirect gas-chromatographic determination of IPC and CIPC in potato-tubers, Nahrung-Food, 29**,** 1985, 487-490.

[25]. W. H. Gutenmann and D. J. Lisk, Herbicide residues - electron affinity residue determination of CIPC, monuron, diuron and linuron by direct hydrolysis and bromination, Journal of Agricultural and Food Chemistry,12**,**1964, 46-48